sequence chi (5′-GCTGGTGG-3′). The chi sequence occurs roughly once every five kb in the E. coli genome, suggesting that RecBCD must travel for long distances along genomic DNA.
In our assay, we observe the enzymatic activity of RecBCD on individual DNA molecules. Fluorescently labeled RNA polymerase and hydrolytically inactive EcoRI(E111Q) were selected as model roadblock proteins. By preparing a DNA substrate with these fluorescently labeled proteins, we directly observed the outcome of collisions with RecBCD. Our results indicate that RecBCD is able to push and eventually displace multiple proteins without reducing its rate of translocation. These results offer the first direct observation of collisions between a helicase and other proteins along the same DNA helix. We propose that the highly processive, dual motor structure of RecBCD is necessary for stimulating recombination many thousands of bp away from the initial dsDNA break. Our results provide additional evidence that an essential, if underappreciated, aspect of helicase function is the ability to clear dsDNA for further processing by other enzymes.

329-Pos
The DNA-Gate of Gyrase Is Predominantly in the Closed Conformation During DNA Supercoiling
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DNA topoisomerases catalyze the inter-conversion of DNA topoisomers and impact key cellular events such as replication, recombination, and transcription. Gyrase catalyzes the introduction of negative supercoils into DNA *via* a strand-passage mechanism. In the first step, a DNA-segment, the gate-DNA, binds to gyrase. The gate-DNA is cleaved, and a covalent DNA-gyrase complex is formed. A second DNA segment, the transfer-DNA, is passed through the gap, and the gate-DNA is re-ligated. Strand passage requires opening of a transient protein interface at the cleavage site, the so-called DNA-gate, by ~2 nm. The intermediate cleavage complex presents an inherent danger of double strand DNA breaks and thus genome instability, and cleavage complexes have consistently been detected in very low amounts. In contrast, a recent study predicted frequent opening of the topoisomerase II DNA-gate. Here, we present a single molecule FRET study that monitors both the conformation of DNA bound to the DNA-gate of gyrase, and the conformation of the DNA-gate itself. DNA bound to gyrase adopts two different conformations, one slightly, one severely distorted from B-DNA geometry. Distortion requires cleavage, but neither ATP nor a transfer-DNA. The DNA-gate of gyrase is predominantly in the closed conformation, in agreement with <5% of cleavage complexes in equilibrium. Importantly, gyrase with an open DNA-gate is also not significantly populated during the relaxation and supercoiling reactions. Presumably, distortion of the gate-DNA unlatches the DNA-gate, and prepares it for transient release by the transfer-DNA, thus providing a strict coupling of gate-opening to strand passage.

330-Pos
Investigating the Nucleation and Extension Rates of E.coli and Deinococcus RecA Along Duplex DNA
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RecA is a protein which promotes the exchange between two homologous DNA molecules in homologous recombination process. When individual RecA molecules assemble on DNA, the DNA is stretched and underwound to form a nucleoprotein filament with its rigidity and end-to-end length increased. We have developed single-molecule tethered particle motion (TPM) experiments to study the assembly dynamics of RecA proteins on individual duplex DNA molecules. The TPM method is capable of measuring the changes in DNA length by observing the bead's Brownian motion, thus allowing us to monitor RecA nucleation and extension in real-time. Using much shorter DNA (a few hundreds basepairs), TPM experiments offer improved sensitivity, since the DNA length change can be readily detected as soon as a few RecA bounded to duplex DNA molecules. Our experiments indicated a faster nucleation rate compared to the previous reports (Galletto et al., 2006). Moreover, we have compared the nucleation and extension rates of E. coli RecA with the RecA from Deinococcus radiodurans, UV-resistant bacteria, under different nucleotide states, ATP and ATPγS. Deinococcus radiodurans RecA are found to nucleate faster (~1.6×10-2 bp-1min-1) but extend slower (~0.3-1.5 RecA/sec) under ATP.This difference reflects the physiological role of Dr. RecA when extensive UV-damaged DNA molecules are present.

331-Pos
Effect of DNA Supercoiling on DNA Decatenation and Unknotting Followed By Brownian Dynamics Simulations
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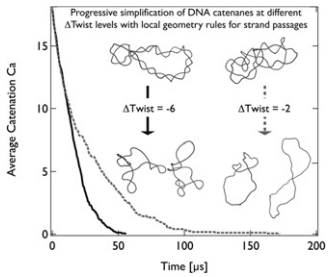
Replication of circular DNA proceeds through a stage of multiply interlinked catenanes that have to be rapidly spatially separated. In addition, knotting of circular DNA has also to be avoided. In bacteria, topology simplification requires participation of two type II topoisomerases: gyrase and topo IV. Several simulation approaches were applied to explain the very efficient topology simplification in that system. Mainly two strategies were explored: in the first one, the system follows its free energy gradient influenced by supercoiling, and in the second one, specific geometrical rules are defined for the selection of strand passages (hooking, chirality). The Monte-Carlo methods usually used to estimate the efficiency of these strategies do not allow to follow DNA topology simplification dynamically, to evaluate its speed, for example. To overcome this limitation, we simulated DNA unknotting and decatenation by Brownian dynamics, which allows for a natural integration of the strategies mentioned above. By following the topological state of the simulated DNA chains (see figure), we show that the combination of supercoiling and local geometrical selection rules provides an important drive for unknotting and decatenation, especially at low topological complexity.



332-Pos
Does T7 DNA Polymerase Backtrack During Proofreading?
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DNA replication is an essential cell process in which the genetic information is copied by replicative DNA polymerases (DNAp). The molecular basis of DNA replication is the addition of nucleotides by DNAp to a growing primer, using single-stranded DNA as a template. High fidelity of the processive T7 DNA polymerase comes from nucleotide selection at the polymerase active site, but is increased several orders of magnitude by an additional intrinsic proofreading ability. In this kinetic process, a partly melted primer shuttles to the exonuclease active site where incorporated mismatches are excised. After excision of erroneous nucleotides, the trimmed primer can shuttle back to the polymerase active site to resume replication. Elucidating the mechanism of the shuttling between these two activities of DNAp is essential for understanding the proofreading mechanism of DNA polymerases.
Transfer of the primer to the exonuclease active site is induced by disruption of the primer-template structure upon the incorporation of a mismatch. Application of tension to the DNA also destabilizes the primer-template structure and can therefore be used to shift the fine-tuned balance between polymerization and proofreading (Wuite *et al*, 2001; Ibarra *et al*, 2009).
Using optical tweezers, we study the kinetic coordination between exonuclease and polymerase activities, while applying different tensions. In these experiments we observe an additional waiting state between proofreading activities, during which the DNAp remains bound to the DNA. The force-dependent rate out of this state suggests that DNAp enters a state comparable to RNA polymerase backtracked state, which was shown to play a role in tuning the fidelity (Shaevitz *et al*, 2003). We speculate that our observed waiting state might play a similar role in the fidelity of DNA polymerase.

333-Pos
A Single Molecule View of the Rad51-ssDNA Interaction
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Homologous recombination (HR) represents an essential DNA repair mechanism in living cells. The central molecular complex of HR is the nucleoprotein filament, a DNA-protein complex in which recombinase protein Rad51 is bounded onto single-stranded DNA (ssDNA) in a helical form. Earlier studies have shown that efficient filament formation is critical for correct DNA repair, therefore a detailed characterization of the interaction between Rad51 and ssDNA is essential to understanding homologous recombination.

We use a combination of single-molecule fluorescence microscopy, optical tweezers and microfluidics to study the interaction of Rad51 with ssDNA. With this approach, we are able to directly visualize Rad51 filament assembly and disassembly on ssDNA at the single-molecule level.